Molecular epidemiology of *Staphylococcus aureus* strains isolated from inpatients with infected diabetic foot ulcers in an Algerian University Hospital

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Abstract

Staphylococcus aureus is the most common pathogen cultured from diabetic foot infection (DFI). The consequence of its spread to soft tissue and bony structures is a major causal factor for lower-limb amputation. The objective of the study was to explore ecological data and epidemiological characteristics of *S. aureus* strains isolated from DFI in an Algerian hospital setting. Patients were included if they were admitted for DFI in the Department of Diabetology at the Annaba University Hospital from April 2011 to March 2012. Ulcers were classified according to the Infectious Diseases Society of America/International Working Group on the Diabetic Foot classification system. All *S. aureus* isolates were analysed. Using oligonucleotide arrays, *S. aureus* resistance and virulence genes were determined and each isolate was affiliated to a clonal complex. Among the 128 patients, 277 strains were isolated from 183 samples (1.51 isolate per sample). Aerobic Gram-negative bacilli were the most common isolated organisms (54.9% of all isolates). The study of ecological data highlighted the extremely high rate of multidrug-resistant organisms (MDROs) (58.5% of all isolates). The situation was especially striking for *S. aureus* [(85.9% were methicillin-resistant *S. aureus* (MRSA)], *Klebsiella pneumonia* (83.8%) and *Escherichia coli* (60%). Among the *S. aureus* isolates, 82.2% of MRSA belonged to ST239, one of the most worldwide disseminated clones. Ten strains (13.7%) belonged to the European clone PVL+ ST80. *ermA*, *aacA-aphD*, *aphA*, *tetM*, *fosB*, *sek*, *seq*, *lukDE*, *fnbB*, *cap8* and *agr* group 1 genes were significantly associated with MRSA strains ($p < 0.01$). The study shows for the first time the alarming prevalence of MDROs in DFI in Algeria.

Keywords: Algeria, diabetic foot ulcer, ecology, infection, multidrug-resistant organism, oligonucleotide array, *Staphylococcus aureus*
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Introduction

Diabetes mellitus is a serious public health problem that is rapidly expanding worldwide [1]. According to the National Institute of Public Health TAHINA survey, the prevalence of diabetes was around 8% in Algeria in a population ranging from 35 to 70 years [2]. Foot ulcers are a common complication in diabetic patients, with prevalence as high as 15–25% [3]. These ulcers frequently become infected, and spread of infection to soft tissue and bony structures is a major causal factor for lower-limb amputation [4], making early diagnosis and adequate treatment essential. As microorganisms are normally present on skin wounds, diagnosis of infection must be based not on microbiological findings but on symptoms and clinical signs, as emphasized by the Infectious Diseases Society of America (IDSA), the International Working Group on the Diabetic Foot (IWGF) and the French Society for Infectious Pathology [4]. However, due to the confounding effect of neuropathy and ischaemia on local and systemic inflammatory responses, diagnosing diabetic foot infection (DFI) at an early

stage is often difficult, resulting in misuse of antibiotics [4]. Conversely, inappropriate antibiotic usage contributes to the increasing prevalence of multidrug-resistant organisms (MDROs), notably methicillin-resistant *S. aureus* (MRSA) [5–10]. To date very few epidemiological data are available about *S. aureus* strains isolated in diabetic foot ulcers (DFUs) and we are not aware of any such study that was performed in Algeria.

In this country, the European community-acquired MRSA clone ST80-IV harbouring Panton–Valentine leukocidin (PVL) is responsible for more than one-third of both community and hospital infections [11,12]. Considering the high prevalence of diabetes mellitus in Algeria and the few data available about pathogens involved in DFI in this country, we decided to identify bacteria responsible for this condition; more specifically we aimed to determine the prevalence and the genotyping profiles of *S. aureus* from an Algerian hospital setting and to compare our data with those published in other countries. Finally, the identification of the role of MDROs in DFI might help clinicians to choose antibiotic agents in a more rational way.

Materials and Methods

Prospective study

Between 1 April 2011 and 30 March 2012, we prospectively enrolled all diabetic patients admitted to the Department of Endocrinology and Diabetology at Annaba University hospital (Algeria) for DFI. Patients were included if they had not received any antibiotic agents in the previous week. This study was carried out in accordance with the Declaration of Helsinki as revised in 2000. The presence and severity of DFI (grade 2–4) were assessed using the IDSA-IWGDF classification system [4].

After wound debridement, samples for bacterial culture were obtained by scraping the wound base and collecting debris by swabbing the wound base, needle aspiration or tissue biopsy and immediately sent to the bacteriology department.

Patients with PVL+ strains were monitored at 6 months to assess the wound outcome (healing/worsening).

Microbiological study

S. aureus was identified by conventional methods (Gram-positive cocci, catalase positive, mannitol fermenting, ability to coagulate rabbit plasma) (BioMérieux, Marcy-l'Etoile, France) and Pastorex Staph Plus agglutination (BioMérieux). Antimicrobial sensitivity was determined by the disk diffusion method according to recommendations of the Antibiotic Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/pages/?page=746&id_page=182). Susceptibility to methicillin was screened by agar diffusion using cefoxitin disks (BioRad, Marnes-La-Coquette, France). The *S. aureus* strains isolated during the inclusion period were preserved in deep agar gel. To be classified as a multidrug-resistant organism (MDRO), *S. aureus* should be methicillin resistant and Gram-negative bacilli resistant to the third generation cephalosporins (3GC). Those isolates included 3GC-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to ceftazidime.

Oligonucleotide DNA arrays and genotyping

Each *S. aureus* isolate collected during the study was analysed at the INSERM laboratory in Nîmes, France. The Alere StaphyType DNA microarray was used according to protocols and procedures previously detailed [13,14]. The test was performed in 5 h. The DNA microarray covers 333 target sequences, including species markers, SCC*mec*, capsule and *agr* group typing markers, resistance genes, exotoxins and MSCRAMM (microbial surface components recognizing adhesive matrix molecules) genes. Primer and probe sequences have been previously published [13]. The *S. aureus* strains were grown on Columbia blood agar and incubated overnight at 37°C. Culture material was enzymatically lysed prior to DNA preparation using commercially available spin columns (Qiagen, Hilden, Germany). Purified DNA samples were used as templates in a linear primer elongation with one primer per target. All targets were amplified simultaneously and, during this step, biotin-16-dUTP was incorporated into the resulting amplicons. Then amplicons were hybridized to the microarray, washed and blocked before addition of horseradish-peroxidase-streptavidin conjugate. After further incubation and washing steps, hybridizations were visualized as spots using a precipitating dye. A digital picture of the microarray was taken and analysed using a dedicated reader and software (ALERE Technologies, GmbH, Jena, Germany). The affiliation of isolates to clonal complexes (CCs) or sequence types (STs) as defined by MLST [14] was determined by an automated comparison of hybridization profiles with a collection of reference strains previously characterized [13,14]. A CC may be defined as a cluster of strains (clones) that are close enough together to be claimed to share to a common origin.

Statistical analysis

The presence of methicillin resistance in *S. aureus* strains was analysed according to demographic and clinical characteristics of study patients and the repartition of the different virulence genes using Fisher's exact test. Statistical analysis was performed using the S-Plus 2000 software package (Insightful Corporation, Seattle, WA, USA) and results were considered significant for $p < 0.05$.

Results

Clinical data and microbiological considerations

From April 2011 to March 2012, 128 patients were hospitalized for DFI and included in the study. Two hundred and seventy-seven strains were isolated (Table 1) from 183 samples, corresponding to a mean number of 1.51 isolates per sample. A polymicrobial infection was present in 92 samples (six with four bacteria, 17 with three bacteria, 69 with two germs). In 64 samples, infection was monomicrobial and 27 cultures were negative. Aerobic Gram-negative bacilli were the most commonly isolated microorganisms (54.9% of all isolates). Among them, Enterobacteriaceae were the most frequent (43.7% of all isolates), especially *Proteus* sp. (34.7% of enterobacteria). Non-fermentative Gram-negative bacilli were rather uncommon (11.2% of all isolates). Aerobic Gram-positive cocci accounted for 45.1% of all organisms, with *S. aureus* as the most commonly isolated pathogen (n = 85, i.e. 30.7% of the aerobic bacteria and 68% of the Gram-positive cocci). Surprisingly, *Streptococcus* sp. was very rarely detected (0.7% of all isolates). No anaerobes were isolated. A high number of isolates were MDRO (58.5%). MRSA was the most common resistant bacteria (85.9% of *S. aureus*, 45.1% of all resistant isolates). Among Gram-negative bacilli, 57% of enterobacteria were multidrug resistant, with a dramatically high percentage of *Klebsiella pneumonia* (83.8%) and *Escherichia coli* (60%). Moreover all *Acinetobacter baumannii* and more than half of *P. aeruginosa* were MDRO.

As previously mentioned, *S. aureus* was isolated in 85 patients from the bacterial culture of their infected wound. The demographic and clinical characteristics of these patients are shown in Table 2. Twenty-one DFIs (24.7%) were classified as grade 2, 55 as grade 3 (64.7%) and nine as grade 4 (10.6%). In 40 patients (47.1%) the current wound was the first episode of ulceration. Sixteen wounds (18.8%) were associated with osteomyelitis. There was no significant difference in the baseline characteristics of patients regarding whether their wounds were infected by methicillin-sensitive *S. aureus* (MSSA) or MRSA, except that previous antibiotic treatment was significantly more frequent in patients with MRSA strains (p 0.007) (Table 2).

TABLE 1. Bacteriological aetiology of diabetic foot infections

Microorganism(s)	*N* (%)	Multidrug-resistant bacteria[a] (%)
Gram-positive aerobic cocci	125 (45.1)	
Staphylococcus aureus	85 (30.7)	73 (85.9)
Enterococcus faecalis	7 (2.5)	–
Coagulase-negative Staphylococcus	31 (11.2)	–
Streptococcus spp.	2 (0.7)	–
Gram-negative aerobic bacilli	152 (54.9)	
Proteus mirabilis	35 (12.6)	12 (34.3)
Klebsiella pneumoniae	31 (11.2)	26 (83.8)
Escherichia coli	20 (7.2)	12 (60)
Morganella morganii	15 (5.4)	8 (53.3)
Enterobacter cloacae	9 (3.2)	5 (55.5)
Proteus vulgaris	7 (2.5)	4 (57.1)
Providencia stuartii	2 (0.7)	1 (50)
Klebsiella oxytoca	1 (0.4)	1 (100)
Citrobacter spp.	1 (0.4)	–
Pseudomonas aeruginosa	23 (8.3)	12 (52.1)
Acinetobacter baumannii	8 (2.9)	8 (100)

[a]Multidrug-resistant bacteria included methicillin-resistant *S. aureus*, Enterobacteriaceae resistant to third-generation cephalosporin, *P. aeruginosa* or *Acinetobacter baumannii* resistant to ceftazidime.

Antimicrobial susceptibility of *S. aureus*

The *in vitro* activities of antimicrobial agents against the 85 *S. aureus* isolates demonstrated a high level of resistance to all classical antistaphylococcal treatments.

All MSSA isolates were susceptible to pristinamycin, fosfomycin and glycopeptides. Gentamicin was the most active aminoglycoside (41.6%). Regarding MRSA, only glycopeptides were active against all the isolates. Compared with MSSA, a significantly higher percentage of MRSA was resistant to some antimicrobial agents, including aminoglycosides, quinolones, cotrimoxazole and tetracycline (p <0.01).

Clonal complexes (CCs) distribution of *S. aureus*

Using the DNA arrays technology, all the *S. aureus* strains we isolated were analysed. The majority of the 73 MRSA belonged to Brazilian clone ST239 (n = 60, 82.2%). Ten strains (13.7%) were assigned to the European clone ST80 and the last three (4.1%) to a CC unknown in the database.

Regarding MSSA, they belonged to a great diversity of CCs: CC1 (n = 3), CC15 (n = 3), CC121 (n = 2), CC9 (n = 1), CC54 (n = 1) and ST152 (n = 1). One isolate was contained in a CC unknown in the database. Concerning the strains isolated from bone, all were MRSA; 14 belonged to ST239 and two to clone ST80.

Resistance and virulence profiles

The prevalence of resistance and virulence determinants are summarized in Table 3.

Analysis of the resistance genes completely agreed with conventional susceptibility data. All MRSA isolates were detected by the cefoxitin test and were positive for *mecA* and SCC*mec* cassette by DNA arrays. The most prevalent macrolide resistance gene in MRSA strains was *ermA*, which was detected in 39.7% (n = 29) of the isolates, whereas *ermC* was found as a single *erm* gene in 16.6% (n = 2) of MSSA isolates. The aminoglycoside resistance genes were found only in MRSA strains; *aphA* and *aacA-aphD* were the most prevalent genes (95.9% and 90.4% of the isolates, respectively). The tetracycline resistance genes (*tet* efflux genes) were detected

TABLE 2. Demographic and clinical characteristics of study patients

Characteristics	Value[a] Patients with MSSA *n* = 12	Patients with MRSA *n* = 73	Total *n* = 85	p MSSA vs. MRSA
Age (range), years	63 (47–82)	64.5 (23–83)	64 (23–83)	NS
Male/female, *n* (%)	7 (58.3) / 5 (41.7)	46 (63)/27 (37)	53 (62.3)/32 (37.7)	NS
Type 1/Type 2 diabetes mellitus	2/10	16/57	18/67	
Cardiovascular disease				
Absence	4 (33.3)	18 (24.6)	22 (25.9)	NS
Coronary heart disease	0 (0)	8 (11)	8 (9.4)	NS
Peripheral arterial disease	7 (58.3)	46 (63)	53 (62.3)	NS
Arterial hypertension	9 (75)	43 (58.9)	52 (61.1)	NS
Stroke	1 (8.3)	2 (2.7)	3 (3.5)	NS
Nephropathy				
Absence	7 (58.3)	32 (43.8)	39 (45.9)	NS
Microalbuminuria	1 (8.3)	13 (17.8)	14 (16.4)	NS
Proteinuria	2 (16.7)	17 (23.3)	19 (22.3)	NS
Renal failure	3 (25)	15 (20.5)	18 (21.2)	NS
Neuropathy				
Peripheral	10 (83.3)	56 (76.7)	66 (77.6)	NS
Autonomic	0 (0)	5 (6.8)	5 (5.9)	NS
Diabetic retinopathy				
Absence	5 (41.7)	21 (28.7)	26 (30.6)	NS
Non-proliferative diabetic retinopathy	5 (41.7)	41 (56.1)	46 (54.1)	NS
Proliferative diabetic retinopathy	2 (16.7)	11 (15.1)	13 (15.3)	NS
Lifestyle factors				
Obesity	5 (41.7)	15 (20.5)	20 (23.5)	NS
Smoking	2 (16.7)	13 (17.8)	15 (17.6)	NS
Alcoholism	1 (8.3)	2 (2.7)	3 (3.5)	NS
Sedentary	6 (50)	23 (31.5)	29 (34.1)	NS
First wound/ recurrence	7 (58.3)/5 (41.7)	33 (45.2)/40 (54.8)	40 (47.1)/45 (52.9)	NS
Prior antibiotic therapy	8 (66.7)	70 (95.9)	78 (91.7)	0.007
β-Lactams	6 (50)	53 (72.6)	59 (69.4)	NS
Aminoglycosides	1 (8.3)	7 (9.6)	8 (9.4)	NS
Quinolones	2 (16.7)	9 (12.3)	11 (12.9)	NS
Macrolides	0 (0)	12 (16.4)	12 (14.1)	NS
IDSA grade				
2	4 (33.3)	17 (23.3)	21 (24.7)	NS
3	8 (66.7)	47 (64.4)	55 (64.7)	NS
4	0 (0)	9 (12.3)	9 (10.6)	NS
Samples				
Scraping-swabbing	12 (100)	63 (86.3)	75 (88.2)	NS
Needle aspiration	0 (0)	6 (8.2)	6 (7.1)	NS
Tissue/bone biopsy	0 (0)	4 (5.5)	4 (4.7)	NS

NS, not significant.
*Values are numbers and percentages in brackets.

in 98.6% (*n* = 84) of the isolates. Compared with MSSA, MRSA strains exhibited a significantly higher prevalence rate for *ermA*, *aacA-aphD*, *aphA*, *sat*, *tetM* and *fosB* genes (p <0.01). No *van* genes were detected, in agreement with the *in vitro* susceptibility data.

Strains were characterized by some distinctive features regarding the distribution of virulence factors: high prevalence of enterotoxins (*sek* (*n* = 65, 76.5%), *seq* (*n* = 64, 75.3%) and *sea* (*n* = 38, 44.7%)), haemolysins (*hlg*, *hlgv, hlgA, hld*), genes encoding intracellular adhesion proteins (*icaA, icaC, icaD*) and capsular polysaccharide type 8 (*n* = 81, 95.3%), together with four genes encoding MSCRAMM (*ebpS*, *clfA*, *clfB*, *fnbA*). A majority of the isolates were found in *agr* group 1 (*n* = 64, 75.3%). *seb*, *egc cluster*, *seh*, *hla*, *cap5* and *agr* group 2 were significantly more prevalent in MSSA strains and *sek*, *seq*, *lukDE*, *fnbB*, *cap8*, and *agr* group 1 in MRSA strains (p <0.01). Genes encoding exfoliatin A and B toxins were not found. On the other hand, *etD* was detected in ten (11.8%) strains. DNA array analysis revealed that 12.9% (*n* = 11) of isolates were *edinB* positive. In ten strains, *edin-B* and *etD* genes were found to be associated. Genes encoding PVL were detected in 11 MRSA isolates (15%) and in one MSSA isolate (8.3%). All these strains belonged to the CC80-MRSA (European community MRSA Clone). A 6-month follow-up of the patients harbouring these strains demonstrated that all of them had a poor outcome (amputations).

Discussion

This study highlighted the extremely high rate of MDRO (58.5%) in DFI from Algerian patients. The prevalence of MDRO was especially striking for *S. aureus* (85.9% of MRSA among staphylococci), *Klebsiella pneumonia* (83.8%) and *Escherichia coli* (60%). This burden of resistance might be directly associated with DFI because a national survey highlighted that the MRSA rate is 35% in Algeria, increasing to 40.7% for hospitalized patients (www.sante.dz/aarn/documents/pdf/rapport11.pdf). The same trend was found for *K. pneumoniae* (52.5% were resistant to cefotaxime) and *E. coli* (13.4%). Another surprising feature was the predominance of Gram-positive cocci as *S. aureus* is considered to be the most

TABLE 3. Main virulence profiles and resistance determinants of *S. aureus* isolated from diabetic foot ulcers

	MSSA *n* = 12	MRSA *n* = 73	TOTAL *n* = 85	*p* MSSA vs. MRSA
Virulence genotyping				
Enterotoxins				
sea	4 (33.3)	34 (46.5)	38 (44.7)	NS
seb	3 (25)	0 (0)	3 (3.5)	0.002
egc cluster	5 (41.6)	2 (2.7)	7 (8.2)	<0.001
seh	3 (25)	0 (0)	3 (3.5)	0.002
sek	3 (25)	62 (85)	65 (76.5)	<0.001
seq	3 (25)	61 (83.5)	64 (75.3)	<0.001
Other toxins				
tst	0 (0)	0 (0)	0 (0)	NS
etA	0 (0)	0 (0)	0 (0)	NS
etB	0 (0)	0 (0)	0 (0)	NS
etD	0 (0)	10 (13.7)	10 (11.8)	NS
edinA	0 (0)	0 (0)	0 (0)	NS
edinB	1 (8.3)	10 (13.7)	11 (12.9)	NS
lukS-PV/lukF-PV	1 (8.3)	11 (15)	12 (14.1)	NS
lukDE	9 (75)	73 (100)	82 (96.5)	0.02
Haemolysins				
hla	12 (100)	12 (16.4)	24 (28.2)	<0.001
hld	12 (100)	73 (100)	85 (100)	NS
hlgA	12 (100)	73 (100)	85 (100)	NS
hlg	12 (100)	73 (100)	85 (100)	NS
hlgv	12 (100)	73 (100)	85 (100)	NS
MSCRAMM				
bbp	12 (100)	72 (98.6)	84 (98.8)	NS
cna	7 (58.3)	62 (84.9)	69 (81.2)	NS
ebpS	12 (100)	73 (100)	85 (100)	NS
clfA	12 (100)	73 (100)	85 (100)	NS
clfB	12 (100)	73 (100)	85 (100)	NS
fib	10 (83.3)	73 (100)	83 (97.6)	NS
fnbA	12 (100)	73 (100)	85 (100)	NS
fnbB	0 (0)	60 (82.2)	60 (70.6)	<0.001
Capusles				
cap5	3 (25)	1 (1.3)	4 (4.7)	0.008
cap8	9 (75)	72 (98.6)	81 (95.3)	0.008
Other virulent factors				
chp	6 (50)	33 (45.2)	39 (45.9)	NS
scn	12 (100)	72 (98.6)	84 (98.8)	NS
Accessory gene regulator				
agr1	2 (1.6)	62 (84.9)	64 (75.3)	<0.001
agr2	5 (41.6)	2 (2.7)	7 (8.2)	<0.001
agr3	3 (25)	10 (13.7)	13 (15.3)	NS
agr4	3 (25)	16 (21.9)	19 (22.4)	NS
Resistance genes				
mecA	0 (0)	73 (100)	73 (85.9)	<0.001
ermA	0 (0)	29 (39.7)	29 (34.1)	0.004
ermC	2 (16.7)	1 (1.3)	3 (3.5)	0.05
aacA-aphD	0 (0)	66 (90.4)	66 (77.6)	<0.001
aphA	0 (0)	70 (95.9)	70 (82.4)	<0.001
sat	0 (0)	70 (95.9)	70 (82.4)	<0.001
tetM	0 (0)	63 (86.3)	63 (74.1)	<0.001
tet efflux	12 (100)	72 (98.6)	84 (98.8)	NS
fosB	8 (66.6)	64 (100)	72 (84.7)	0.03

NS, not significant.
*Values are numbers and percentages in brackets.

frequently isolated and virulent pathogen in DFI from western countries [15–17]. However, some recent studies have shown that Gram-negative organisms are the most frequent isolates in DFI from patients living in warm climates, especially in India [18–20]. As noted by Lipsky *et al.* [4], there is no clear explanation for this predominance.

The increasing prevalence of antibiotic-resistant bacteria in DFUs, particularly MRSA, both as colonizers or pathogens [6–8], is problematic. MRSA has emerged as a serious and commonly occurring problem in diabetic patients with foot ulcers [5,7]. MRSA requires targeted antibiotic treatment and has been associated with a poor outcome in many reports [9,10,21]. In this study, we focused for the first time on *S. aureus* isolated from DFIs in Annaba University Hospital (Algeria). Even though Gram-negative bacilli as a group were the most prevalent bacteria isolated in our study, *S. aureus* was the most common isolate (Table 1), accounting for 30.7% of all microorganisms, and 85.9% of *S. aureus* were methicillin resistant, in agreement with other studies [5,15,17,22,23]. However, the rate of methicillin resistance is particularly high, ranging from 15 to 30% around the world [24]. While the MRSA prevalence rate is clearly high in Annaba Hospital (62.5% of all *S. aureus* isolated in 2011 were MRSA), it is significantly lower than in strains isolated from DFIs ($p < 0.001$). This high prevalence rate is associated with previous antibiotic treatment in our study and is likely to be attributable to the high proportion of patients with recurrent ulcers (55%): all were previously infected and had been already treated with antibiotics during a preceding hospitalization, a well-established risk factor for selecting antibiotic-resistant organisms, especially in a diabetic foot clinic [3,4,8]. β-Lactams were the most prescribed antibiotic treatment in this population. Potential sources of acquisition of MRSA may include prior hospitalizations and transmission by healthcare providers at home during dressing changes. Even if MRSA is considered to be a colonizer, we could observe in this population that all the osteomyelitis was due to MRSA strains, suggesting that if a rapid debridement is not performed all the strains are a source of serious complications in chronic wounds.

DNA microarray is a technique from molecular biology that is able to detect genes related to virulence and antibiotic resistance [13,14]. Using this method, we were able to compare the strains, determine their clonality and evaluate the virulence and resistance profiles of *S. aureus* [13,14]. In our study, 82.2% of the MRSA isolates we tested belonged to ST239; this clone is considered to be one of the most worldwide disseminated clones [25], being extensively described as a major clone in many countries in Asia, Europe, South America [26] and Africa [27]. It has not been described in Algeria to date. Interestingly, in this study we highlighted the importance of strains harbouring Panton–Valentine leukocidin-encoding genes ($n = 12$, 14.1%). These genes coding for a cytotoxin are claimed to be a major threat in severe tissue necrosis [28] in some but not all previously published studies. This high prevalence is unknown in this pathology, where these strains are rarely isolated from chronic wounds [22,23] and their pathogenicity is low in this setting [29]. In this study, all PVL+ MRSA stains belonged to CC80; this clone has been considered to be the main clone associated with PVL in Europe [30], and also in Algeria [11] and Tunisia [31]. The high prevalence of this clone in Algeria could explain its detection in DFI. However, more surprisingly, all the patients harbouring PVL+ strains had a worse outcome, a result not found in a previous study [29]. Differences in wound care and treatment

compliance or indications for lower-limb amputations could explain these differences.

One of the limitations of the study is the high number of swab samples (93%) but these samples were obtained after scraping the base of the ulcers, as recommended by the IWGDF [4]. Moreover, the number of isolates per sample was low, suggesting that the sampling method was rather in adequate [32].

In conclusion, this study highlighted for the first time the bacterial ecology of DFI in an Algerian hospital, showing a high prevalence of MDRO, notably MRSA strains and a high proportion of Gram-negative bacilli. These features appear close to those previously described in India [18–20]. This trend is alarming and could indicate a misuse of antibiotic agents and/or high prevalence of cross-transmission of microorganisms. Improving knowledge of the local ecology of DFI in Annaba looks to be important for a more rational empirical prescription of antibiotic agents in Algeria if such results are confirmed by further studies in other Algerian areas.

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Transparency Declaration

Nothing to declare.

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